

## Protocol for RNA synthesis with NEB HiScribe T7 Quick High Yield RNA Synthesis Kit

Thaw the necessary kit components, mix and pulse-spin in microfuge to collect solution to the bottoms of tubes. Keep on ice.

Assemble the reaction at room temperature in the following order:

Nuclease-free water	X $\mu$ l
NTP Buffer Mix	10 $\mu$ l (6.7 mM each NTP final)
Template DNA	X $\mu$ l (1 $\mu$ g)
T7 RNA Polymerase Mix	2 $\mu$ l
Total reaction volume	30 $\mu$ l

Mix thoroughly and pulse-spin in a microfuge. Incubate at 37°C for 2 hours.

To remove template DNA, add 30  $\mu$ l nuclease-free water to each 20 $\mu$ l reaction, followed by 2 $\mu$ l of DNaseI (RNase-free), mix and incubate for 15 minutes at 37°C.

**Reference: NEB HiScribe T7 Quick High Yield RNA Synthesis Handbook**